Probing Uranium Resistance by the Aerobic Aquatic Bacterium *Caulobacter crescentus*

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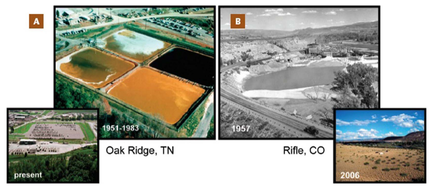
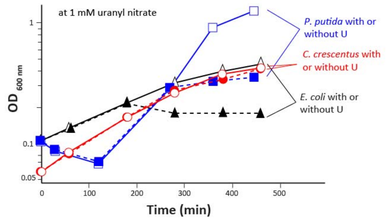
C. crescentus is highly resistant to uranium:

- Uranium (U), particularly in its water soluble form U(VI), poses a significant threat to human health and wildlife as an environmental contaminant [1].
- The remediation of some 120 U-contaminated sites covering 7280 km^2 in the United States has been the focus and responsibility of the DOE. These sites have U(VI) levels up to 2000 times higher than the EPA regulated level of 0.03 mg/L (0.1 µM) [1].
- One strategy to help remediate U contamination is to use microbes which are highly tolerant of U(VI) and are able to mineralize U under aerobic conditions [2].
- The aerobic, aquatic, freshwater bacterium *Caulobacter crescentus* has been shown to be highly tolerant of U(VI). Our aim is to understand U detoxification and biomineralization processes in *C. crescentus* [3-4].





Adapted from Hu et. al. (2005) *J. Bacteriol.* **187**, 8437.

S-layer protective against U stress?:

What is the S-layer?:

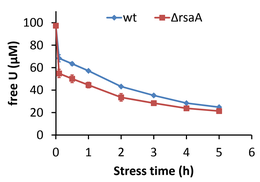
- The S-layer is the outermost layer of the cellular envelope for many bacteria and archaea.
- It consists of protein (RsaA in *C. crescentus*) or glycoprotein that self-assemble into an array on the bacterial surface [5].

S-layer mutant (Δ*rsaA*) is more susceptible to U compared to wt:

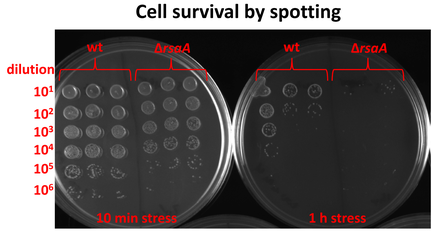
U stress challenge:

1. Wt and Δ*rsaA* were grown in PYE medium to log phase and washed.
2. Cells were re-suspended in 100 µM uranyl nitrate in 10 mM Tris pH 7.0 and incubated at 30 °C with rocking.
3. CFU were determined by serial dilution followed by spotting/colony counting.
4. The amount of U bound to the cells was determined using the ArsenazoIII assay.

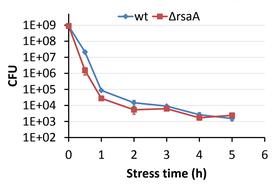
Cellular U absorption



Cell survival by spotting



Cell survival by colony counting



Conclusions:

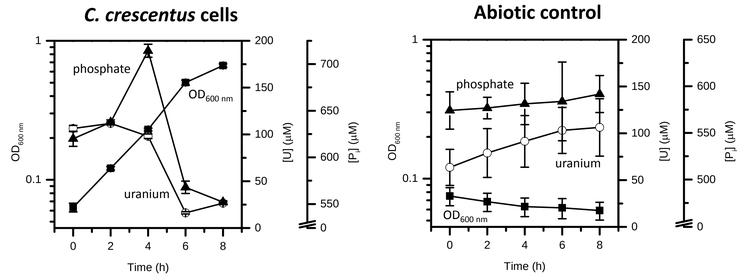
- Wt initially survives better than the S-layer mutant during the U stress challenge at pH 7, suggesting that the S-layer does have a somewhat protective role in U tolerance.
- Cellular U absorption is positively correlated with survival in the stress assay.

Future direction:

- Perform whole cell FTIR and TEM of the cells under the U stress challenge to determine if there is a difference in the U binding mode between mutant and wt.

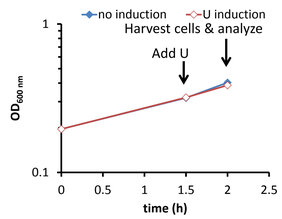
Phosphate metabolism facilitates U precipitation:

C. crescentus cells precipitate U and P_i concurrently during growth in PYE supplemented with 200 µM uranyl nitrate:

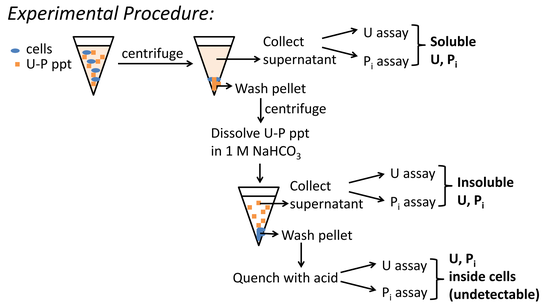


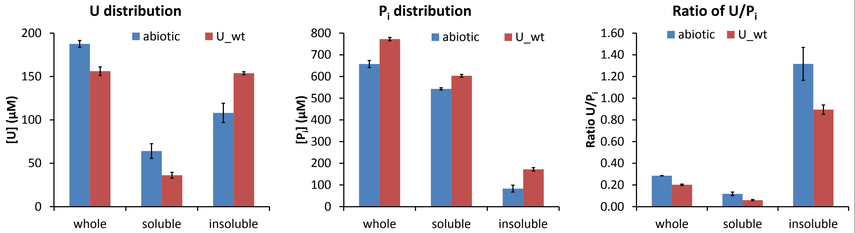
C. crescentus cells produce crystalline U-P minerals in higher amounts compared to the abiotic mineralization process:

U induction during growth:



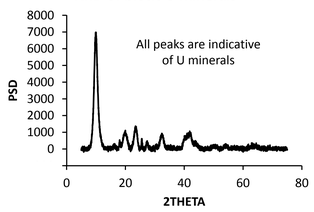
Experimental Procedure:





- Higher amounts of U-P minerals are produced biotically than abiotically.
- The ratio of U/P_i in the biotic minerals is lower than the abiotic minerals.

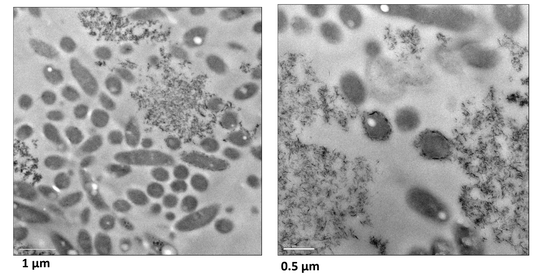
XRD of biotic U minerals

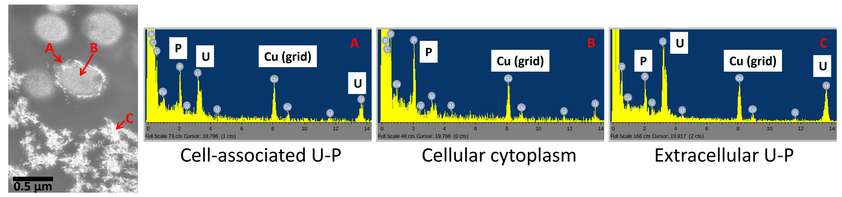


Courtesy of Dr. Mavrik Zavarin (LLNL)

- Crystalline U-P minerals were not detected by XRD in an abiotic control or in a dead cell control.
- XRD analysis indicates the presence of:
  $(NH_4)(UO_2)(PO_4)\cdot 3H_2O$ (uramphite), $K(UO_2)(PO_4)\cdot 3H_2O$ (potassium uranyl phosphate hydrate), and/or $Na_{0.43}K_{0.57}(UO_2)(PO_4)\cdot xH_2O$ (potassium sodium uranyl phosphate hydrate)

TEM coupled with EDS shows that the majority of the U is extracellular:

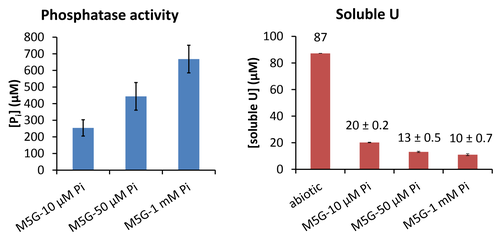


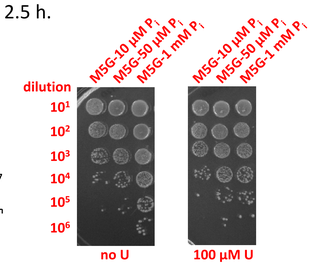


- No significant amount of U is found in the interior of the *C. crescentus* cells.

Cellular phosphatase activity is correlated with cell survival?

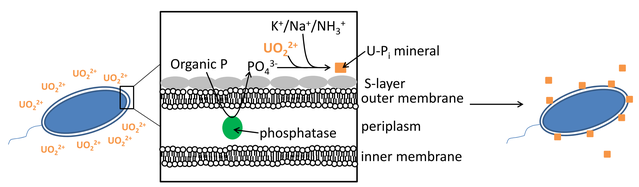
- *C. crescentus* cells were grown in minimal medium (M5G) supplemented with 10 µM (very limited), 50 µM (limited), or 1 mM (excess) P_i.
- U stress challenge at 100 µM uranyl nitrate was performed in the presence of 5 mM β-glycerophosphate as an organic phosphate source for 2.5 h.





- There is a general growth defect with decreasing $[P_i]$.
- There does not appear to be a significant correlation between level of phosphatase activity and survival at 50 and 100 µM U beyond the general growth defect.
- Future studies at higher [U] will be performed to determine if there is a correlation at higher [U].
- The presence of β-glycerophosphate in the U stress challenge improves survival. (Almost no colonies survive at 100 µM U without β-glycerophosphate).

Potential model for U defense in *Caulobacter crescentus*:



References:

[1] EPA US.
[2] NABIR Rep (2003), **LBNL-42959**.
[3] Hu P et. al. (2005) *J Bacteriol*, **187**, 8437-8449.
[4] Hillson NJ et. al. (2007) *Appl Environ Microbiol*, **73**, 7615-7621.
[5] Beveridge TJ et. al. (1997) *FEMS Microbiol Rev*, **20**, 99-149.

Acknowledgement:

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